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TITLE: Development of a Sustained Antiplaque, Antimicrobial Delivery System for KSL Localized in the Oral Cavity

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Development of a Sustained Antiplaque Antimicrobial Delivery System for KSL Localized in the Oral Cavity

This report summarizes the progress since the beginning of research in February. Meetings were held with Dr. Kai Leung and Army personnel in February at the University of Kentucky and the chewing machine was delivered. Preformulation studies commenced and the Analytical Methodology was developed for KSL.

Stability assessment in buffered solutions at pH 4.0, 7.4 and 9.0 reveals excellent stability at 37°C through 7 days at all pHs. The robustness is demonstrated even at 55°C for more than one day at pH 7.4. In artificial saliva, pH 5.7, there was no detectable loss at 37°C for 3 days. Studies are underway in whole mucosal saliva and simulated gastric and intestinal fluids.

Concurrent with the stability studies, since retention of the KSL in the oral cavity for a sufficient period of time will depend on incorporation in polymeric microspheres, retention of the microspheres in the gum base will be assessed. These studies will be done with blank microspheres in a gum base provided.

Incorporation of KSL into microspheres as well as determining the binding of KSL to blank microspheres will be performed. Following these studies, KSL containing microspheres will be incorporated into the gum base and release studies undertaken.

A summary of the results to date follows. In addition to the PI, personnel working on the grant during this period of time were:

Dr. Dong Hee Na
Paolo Blasi
Dr. Yilmaz Capan

DEVELOPMENT OF A SUSTAINED ANTIPLAQUE, ANTIMICROBIAL DELIVERY SYSTEM FOR KSL LOCALIZED IN THE ORAL CAVITY
May 3, 2004

Studies being carried out within the context of the project

Phase I : Preformulation Studies

A- Analytical Methods Development

a. Active Ingredients

Analytical procedures to assess drug content, stability and release have been developed.

Stock solution: KSL 3.0 mg/mL in D.W.
Test concentration: KSL 200 μg/mL in each solution

Analysis: Reversed-phase HPLC with C-18 column
HPLC conditions:
- Column: Prosphere C-18 (4.6 x 250 mm, Alltech, Deerfield, IL)
- Flow rate: 1.2 mL/min
- Injection volume: 40 μL
- Mobile Phase A: Water with 0.1 % TFA
- Mobile Phase B: Acetonitrile with 0.1 % TFA
- Gradient: 80% A & 20% B to 70% A & 30% B in 8 min.
- Detection: UV 215 nm

B- Stability Assessment

1 .pH Effect

KSL was tested at three pH conditions as described below:

pH 4.0, 20 mM sodium acetate buffer
pH 7.4, 20 mM sodium phosphate buffer
pH 9.0, 20 mM sodium borate buffer

2.Temperature Effect

The KSL may be exposed to higher temperatures during fabrication, actual use and storage. Therefore, the stability of the KSL was studied at 25, 37 and 55 °C.

In addition to the stability studies conducted at three different pH values and three different temperatures, stability studies in artificial saliva, simulated gastric fluid (USP), and simulated intestinal fluid (USP) at 37°C will be carried out.

3. Artificial saliva at 37°C

The Artificial saliva was used for the in vitro release study in an attempt to simulate the actual conditions of use. The ingredients of the artificial saliva follows:

Sodium chloride – 0.844 g
Potassium chloride – 1.200 g
Calcium chloride dihydrate – 0.193 g
Magnesium chloride hexahydrate – 0.111 g
Patassium phosphate dibasic – 0.342 g
Water to make to 1 L and pH adjustment with HCl to pH 5.7 ± 0.1

Sampling:

Samples for studies of pH and temperature effects were taken on 0, 1st, 3rd, 7th, 14th, 21st, and 28th days (triplicate). The stability study in artificial saliva was performed for 3 hours.

RESULTS

A. Analytical Method Development

1. HPLC method of KSL

Specificity of HPLC method

Under the described HPLC conditions, the standard of KSL in D.W. was detected as a single peak at the retention time of 7.0 min (Figure 1a). The degradation compounds of KSL produced at each pH conditions (55°C) could be resolved by the HPLC method (Figure 1b-d).

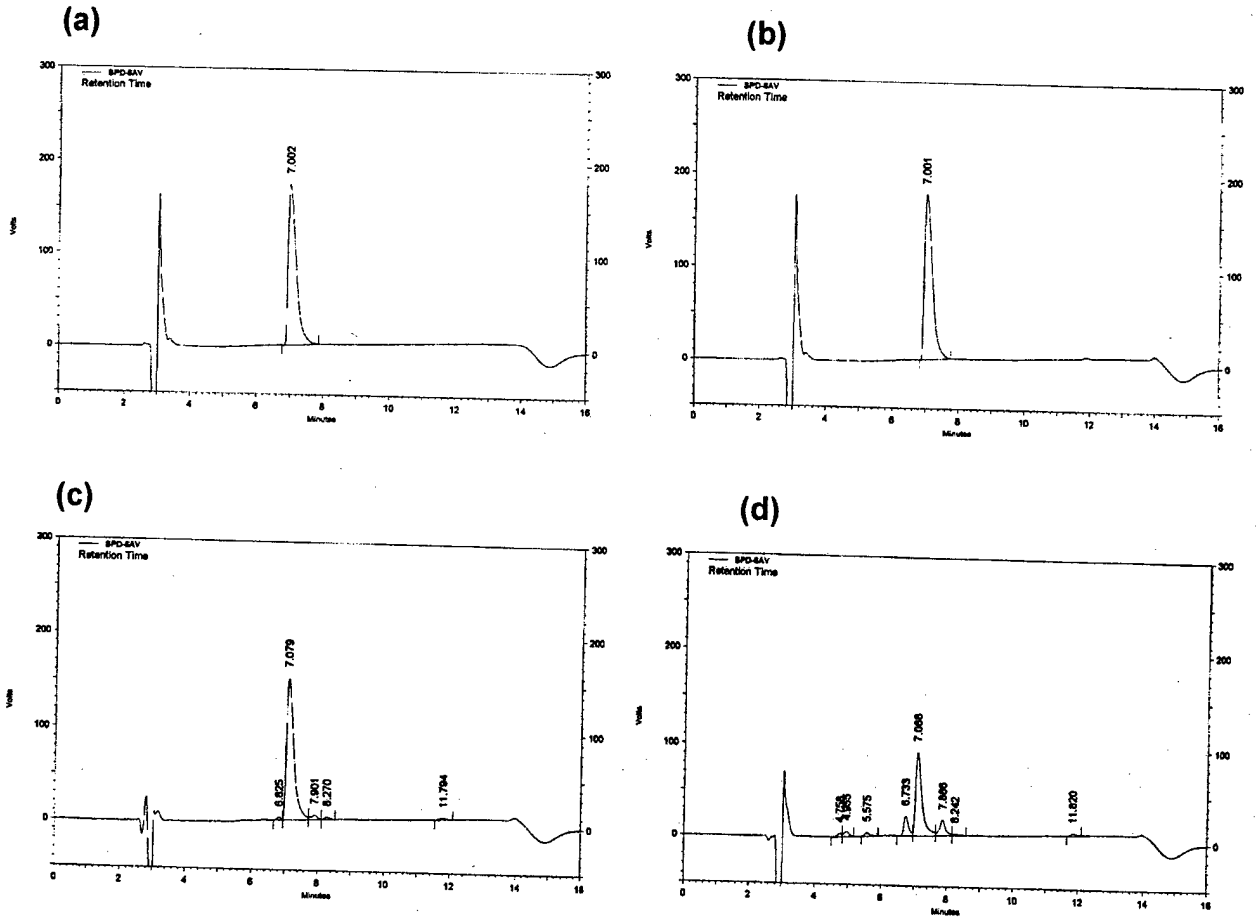


Figure 1. HPLC chromatograms of KSL in various aqueous solutions (**a**: KSL 200 μg/mL in D.W., **b**: KSL incubated at pH 4 (55°C) for 3 days, **c**: KSL incubated at pH 7.4 (55°C) for 3 days, **d**: KSL incubated at pH 9 (55°C) for 3 days).

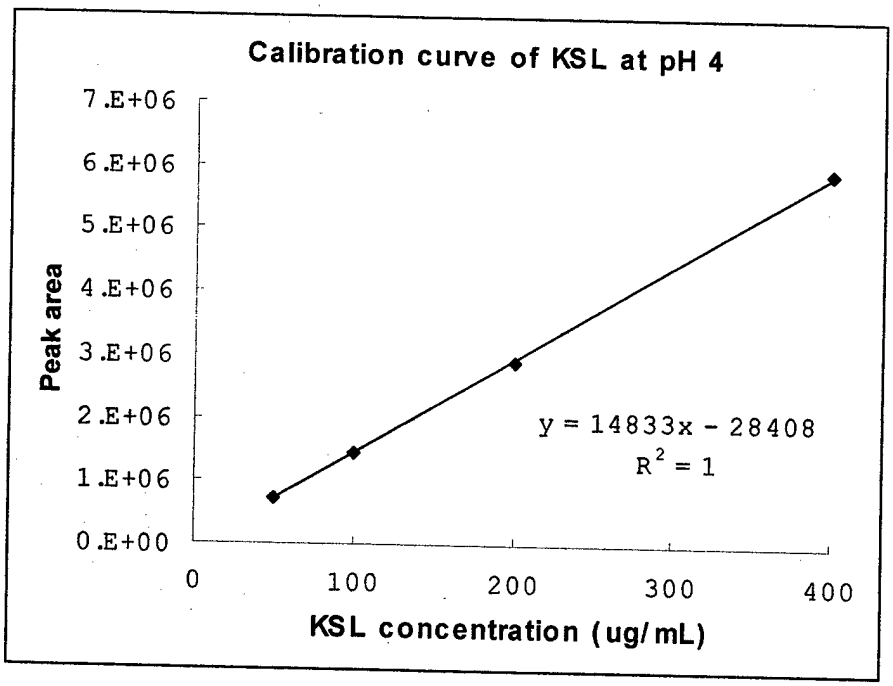
Calibration curve of KSL in each pH solutions

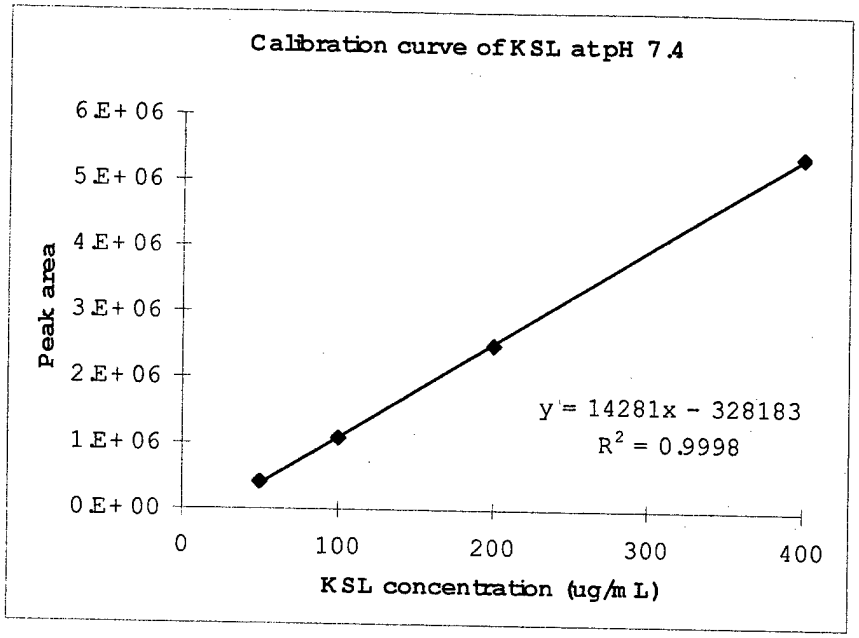
Table 1. Precision and linearity of KSL in various pH solutions

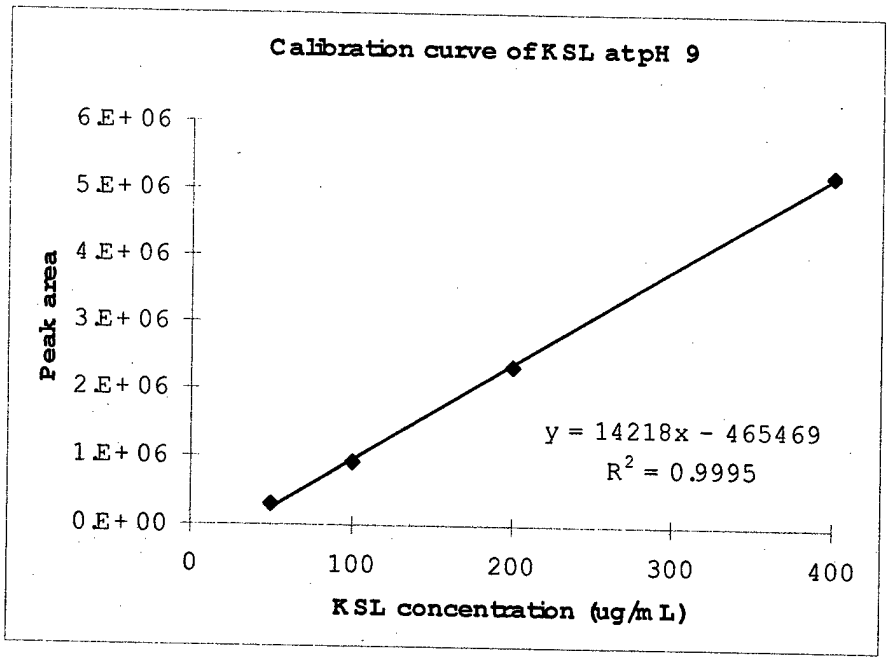
KSL concentration (μg/mL)	pH 4		pH 7.4		pH 9	
	Average peak area[1]	RSD (%)[2]	Average peak area[1]	RSD (%)[2]	Average peak area[1]	RSD (%)[2]
50	725063	5.9	413753	10.1	301632	7.5
100	1455535	2.3	1089352	5.0	918281	5.3
200	2916429	0.7	2495015	1.8	2337003	3.3
400	5913898	2.5	5399814	1.6	5244962	2.8
Linearity	0.9999		0.9998		0.9995	

[1]n=3 (inter-day)

[2]Relative standard deviation

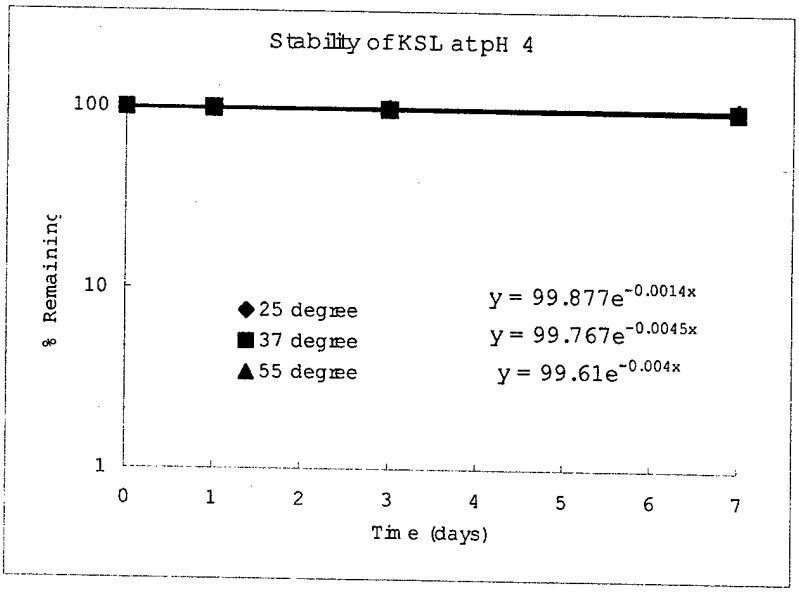


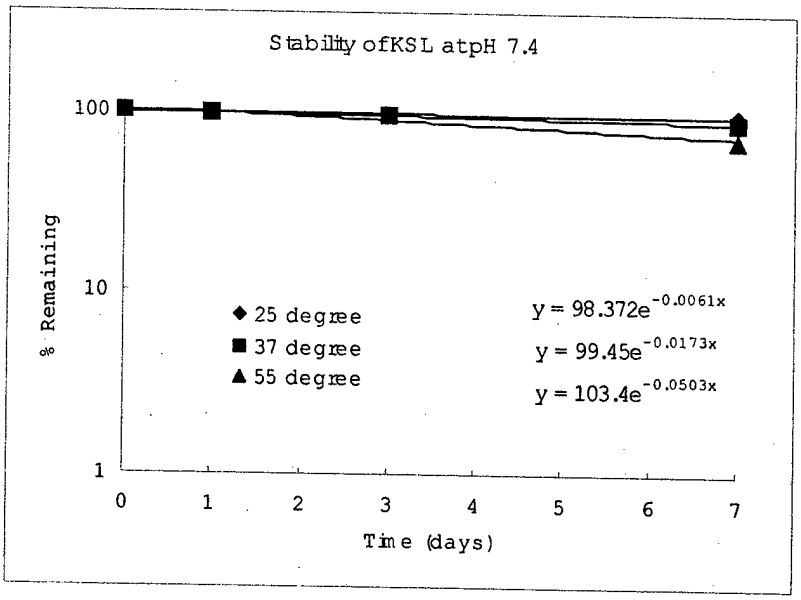

Calibration curve of KSL at pH 7.4
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
Peak area
y = 14281x - 328183
R2 = 0.9998
0
100
200
300
400
KSL concentration (ug/mL)

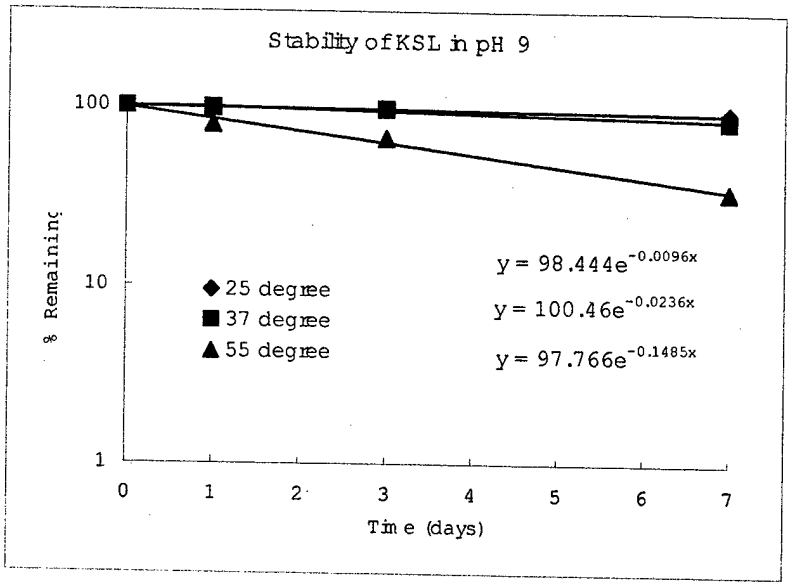

Calibration curve of KSL at pH 9
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
Peak area
y = 14218x - 465469
R2 = 0.9995
0
100
200
300
400
KSL concentration (ug/mL)

B. Stability of KSL in aqueous solutions

1. Degradation rate of KSL at various pH solutions






Stability of KSL in pH 9
100
10
1
% Remaining
25 degree
37 degree
55 degree
y = 98.444e^-0.0096x
y = 100.46e^-0.0236x
y = 97.766e^-0.1485x
0
1
2
3
4
5
6
7
Time (days)

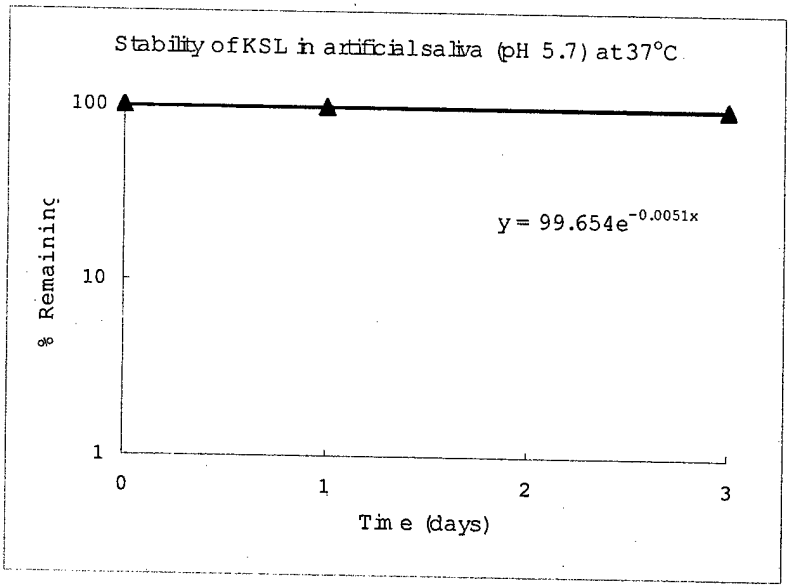
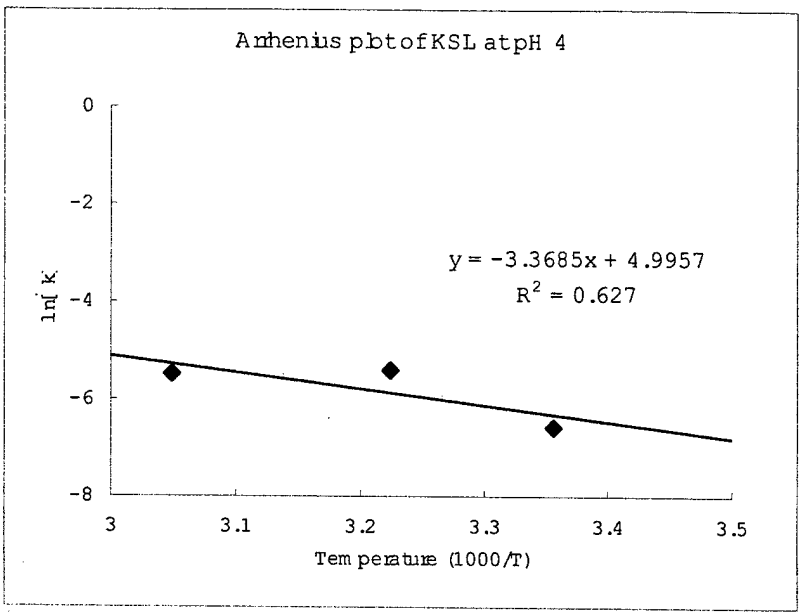

Stability of KSL in artificial saliva (pH 5.7) at 37°C
100
10
1
% Remaining
y = 99.654e^-0.0051x
0
1
2
3
Time (days)

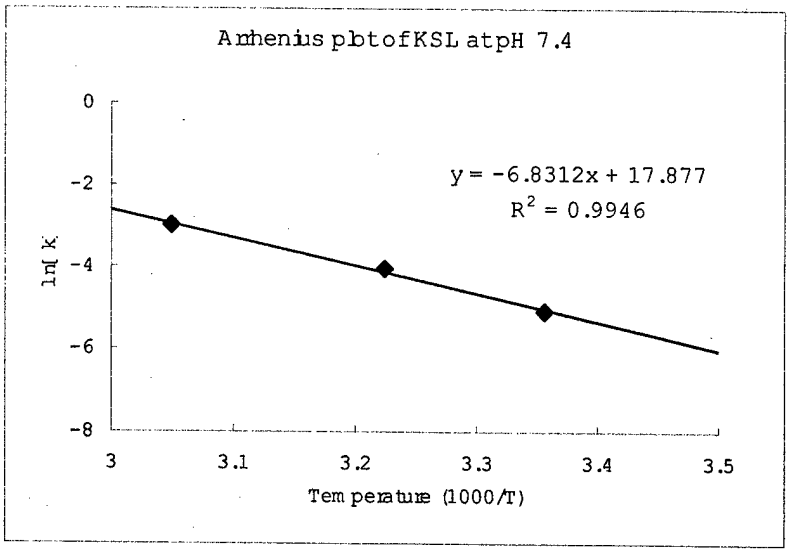
Table 1. Degradation constants of KSL

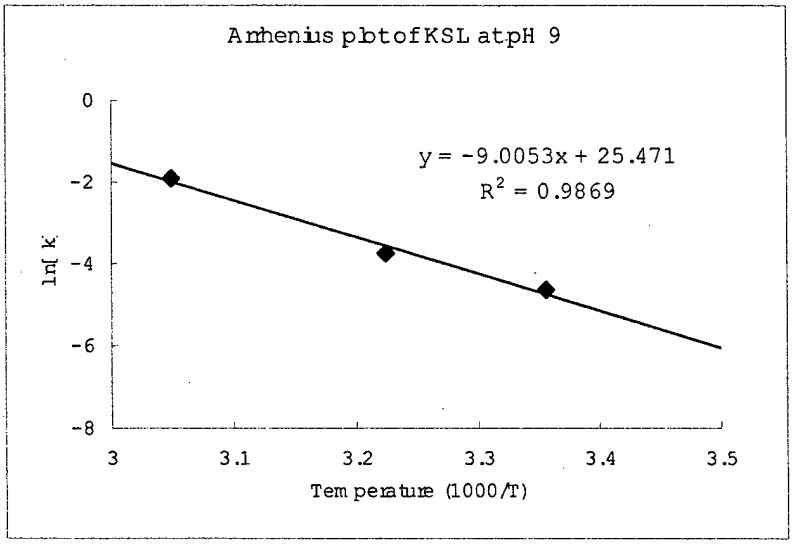
Temperature	pH 4	pH 7.4	pH 9	Artificial saliva
25°C	1.4×10^{-3}	6.1×10^{-3}	9.6×10^{-3}	-
37°C	4.5×10^{-3}	17.3×10^{-3}	23.6×10^{-3}	5.1×10^{-3}
55°C	4.2×10^{-3}	50.3×10^{-3}	148.5×10^{-3}	-
Ea (cal/degree/mole)[1]	6.693	13.574	17.894	-

[1]Activation energy

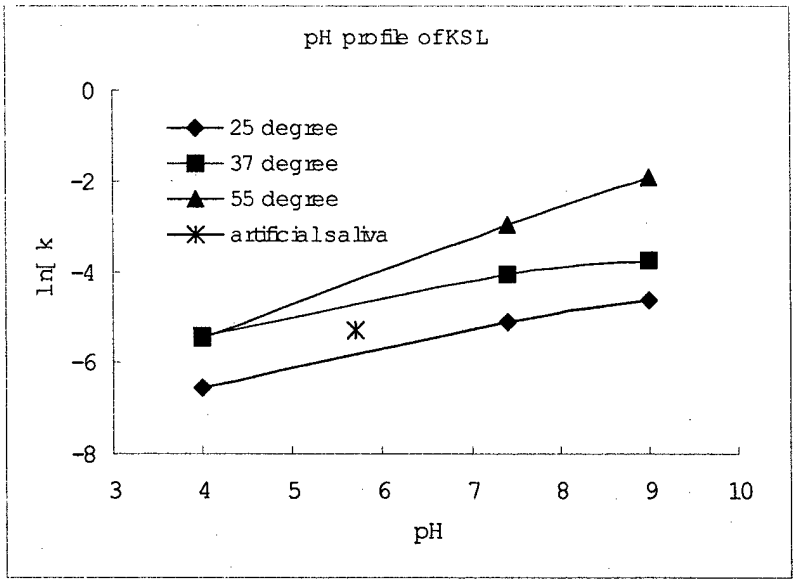
2. Arrhenius plot







3. pH effect on stability of KSL



SCHEDULED STUDIES

A. STABILITY STUDIES

1. Whole mucosal saliva. This has been provided by Dr. Leung
2. Simulated gastric and intestinal fluid

The formulations of the simulated gastric and intestinal fluids:

- *Simulated gastric fluid (USP)*
1 N HCl – 7 mL
NaCl – 2 g
Pepsin – 3.2 g
Water to make to 1 L, pH 1.2

- *Simulated intestinal fluid (USP) at 37°C*
Monobasic potassium phosphate – 6.8 g
Water – 500 mL
0.2 N NaOH – 190 mL
Pancreatin – 10 g
Water to make to 1 L, pH 6.8

Sampling: The stability study in simulated gastric fluid and intestinal fluid will be performed for 12 hours.

B. RETENTION OF MICROSPHERES

Incorporation of blank microspheres into Gum Base
On the basis of a 800 mg gum base, the following mixtures will be prepared.

10 % microspheres; 80 mg microspheres, 720 mg gum base
20 % microspheres; 160 mg microspheres, 640 mg gum base
40 % microspheres; 320 mg microspheres, 420 mg gum base

The mixtures will be subjected to the *chewing apparatus*

C. KSL ADSORPTION AND INCORPORATION INTO MICROSPHERES

C1-Adsorption Studies

Following the preparation of PLGA (Resomer 502 H) microspheres by a solvent extraction/ evaporation process, KSL will be adsorbed to the microsphere surface. Then, the amount of KSL adsorbed to the microsphere surface will be determined as *mg KSL/ mg microspheres*.
Maximum adsorption will be determined and release studies will be carried out in artificial saliva.

C2-Incorporation studies

KSL will be incorporated into microspheres during the fabrication of the microspheres at target drug loads of 10 and 20 % by a solvent extraction/evaporation process, and the actual drug content determined by HPLC. Release studies will be performed in artificial saliva.

D. PREPARATION OF KSL CHEWING GUM

The KSL-adsorbed and KSL-incorporated microspheres as well as KSL-containing chewing gum formulations will be prepared, and characterized for surface morphology by SEM and in vitro release in a buffer solution.

Calculations for determining KSL content:

Total weight of one chewing gum base is 800 mg.
If residence time for chewing gum is taken as 20 min,

Total saliva for 20 min: ~ 50 mL
ED_{99} of KSL: 6.25 μg/mL (Ref. Concannon et al., J. Med. Microbiol., 2003, 52, 1083-1093)
6.25 μg/mL x 50 mL = 312.5 μg KSL required (minimum)

Calculation example:

If drug loading: x 10

312.5x10 = 3125 μg or 3.125 mg KSL

At a load of: 10% (drug/microsphere)

31.25 mg of microsphere (28.125 mg polymer + 3.125 mg KSL) will be added into 1 chewing gum.

** We will start with a minimum of 31.25 mg /chewing gum and test up to a maximum amount that the chewing gum base can hold.

The chewing gum formulations designed for testing

A. Conventional chewing gum formulations

Ingredient	A1 Weight (%)	A2 Weight (%)	A3 Weight (%)
Gum base	52.6	29	30.5
Paraffin oil	3.4	-	-
Powdered sorbitol	40.8	43	-
Sorbitol solution 70%	-	21	-
Mannitol	0.9	-	-
Saccharin	0.1	-	-
Powdered sugar	-	-	50
Corn syrup	-		18
Aspartame	-	0.33	-
Glycerine	-	5	-
Lecithin	-	0.5	0.3
Flavor	2.1	1	1
Coloring	-	-	0.2
Sodium fluoride	0.1	-	-